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Short Communication

Simultaneous pH and ionic strength effects and buffer selection in capillary electrophoretic techniques

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ABSTRACT

Under certain conditions, a decrease in electroosmotic flow can be observed when the pH is increased at constant buffer concentration. This unusual behaviour is related to changes in the ionic strength caused by the titrant.

INTRODUCTION

During optimization of the resolution in the analysis of steroid esters by micellar electrokinetic chromatography [1], it was observed that an increase in pH caused a decrease in the electroosmotic mobility. The result, obtained by using a statistical design technique in which several parameters were varied at the same time, has been confirmed experimentally. This contradicts the general ideas about the dependence of mobility on pH [2–7]. This phenomenon has practical consequences as resolution in both capillary zone electrophoresis [8] and micellar electrokinetic chromatography [9] is influenced by the electroosmotic mobility.

THEORY

A potential difference orginates from the charge separation between a capillary wall and a solution. In fused-silica capillaries, this can be ascribed to the dissociation of surface silanol groups, leaving an excess of negative charges on the wall. Owing to the presence of ions in the solution, the potential difference decays rapidly. At a small distance from the wall, at the shear plane, beyond tightly absorbed ions and solute molecules, the potential difference attains its electrokinetically relevant value, the ζ-potential. The electroosmotic mobility μ_{eo} is proportional to this ζ-potential [10]:

$$\mu_{eo} = \varepsilon_0 \varepsilon_r \zeta / \eta$$

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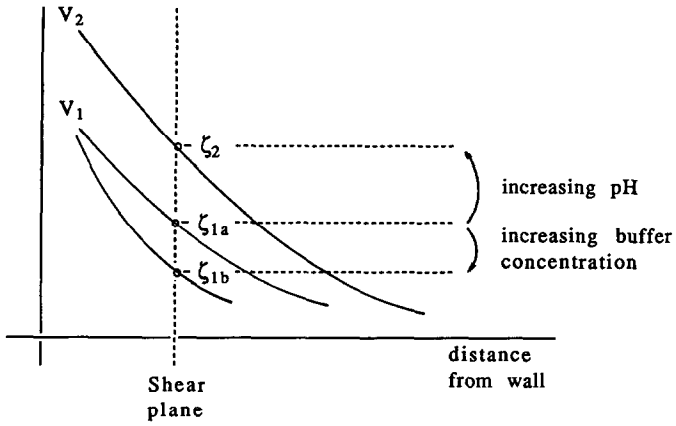

Fig. 1. Influence of buffer pH and concentration on the ζ-potential. See Theory for details.

where μ_{eo} = electroosmotic mobility ($m^2/s \cdot V$), ζ = zeta-potential (V), ε_0 = permittivity of vacuum (8.85 $C^2/N \cdot m^2$), ε_r = dielectric constant and η = viscosity ($kg/m \cdot s$).

Using a simple model, and ignoring the subtle differences between the Debye–Hückel, Goüy–Chapman or Stern treatments [11,12], the effect of buffer composition on the ζ-potential is shown in Fig. 1. Let the surface potential V_1 and the ζ-potential ζ_{1a} represent a reference situation. By increasing the pH of the buffer, the charge on the wall is increased, increasing the surface potential from V_1 to V_2, and resulting in a higher potential ζ_2, as observed at the shear plane. When the buffer concentration, or more precisely the ionic strength, is increased, a given potential V_1 will decay faster, resulting in a decreased potential ζ_{1b}. As represented here, it is assumed that changing the buffer concentration does not affect the surface charge density on the wall. Depending on the case, this may not be a realistic assumption.

Of the two factors, pH and buffer concentration, the latter has been less intensively studied, at least within the context of capillary electrophoretic techniques. Electroosmotic mobilities, or equivalent linear velocities and migration times have been related to buffer concentration [13–17] and ionic strength [17,18]. The studies confirm that, at a fixed pH, mobility decreases with increasing concentration, but confusion exists regarding the mathematical model that should be used to describe this behaviour. VanOrman *et al.* [17] have shown that ionic strength is a more meaningful quantity than concentration.

Many workers have studied the pH dependence of the electroosmotic mobility in fused-silica capillaries [2–6]. The effect has also been studied with micellar solutions [7]. Many more data can be found, hidden in graphs and tables relating analyte mobilities (or equivalent migration times) to pH, as often methanol or another neutral marker is included. It is now generally accepted that the electroosmotic mobility increases strongly up to pH 7–8. At higher pH, the mobility still increases, but more slowly. This continued increase at higher pH does not seem to be universally valid. Apart from our own results (Fig. 2), five other examples were found in the literature where the reverse occurred. Fujiwara and Honda [13,19] reported a decrease in mobility between pH 6 and 10, using a phosphate buffer [13,19]. They ascribed this

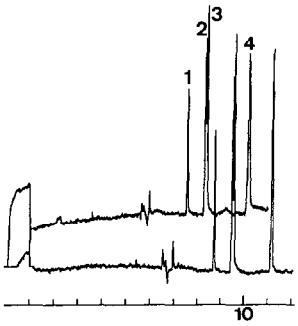

Fig. 2. Effect of buffer pH on electroosmotic flow; micellar electrokinetic chromatographic analysis of testosterone esters: 1 = propionate; 2 = phenylpropionate; 3 = isocaproate; 4 = decanoate. Conditions: 440 V/cm, 35°C, 40 mM SDS, 40% acetonitrile 20 m*M* boric acid–borax buffer; pH 8 (top) and 9 (bottom). Time scale in min.

phenomenon to a "decrease in the ζ-potential with pH" [19], which is just a restatement of the same as no important changes in dielectric constant or viscosity are to be expected. Data by Cohen *et al.* [20] showed a decreased mobility when increasing the pH from 7 to 9, using a sodium dodecyl sulphate (SDS) solution with a phosphate–borax buffer, but they did not comment on this fact. Nishi *et al.* [21] showed the same effect in the range 7–9, again with a phosphate-borax buffer, but in a bile salt solution. The phenomenon was not discussed although the data differed from an earlier report [22], where the electroosmotic mobility remained nearly constant. Using the same buffer, a higher electroosmotic mobility at pH 7 was demonstrated with four different bile salts [23].

EXPERIMENTAL

Electroosmotic mobilities in fused-silica capillaries were measured with an integrated and automated capillary zone electrophoresis instrument, the P/ACE System 2000 (Beckman, Palo Alto, CA, U.S.A.). Standard P/ACE capillaries (57 cm × 75 μm I.D.) were used with detection at 50 cm. Before each run they were rinsed with 0.1 *M* NaOH, water, 0.1 *M* HCl, water and running buffer.

Mesityl oxide was used as the marker, injected by pressure (1 s) and detected at 214 nm. Electroosmotic mobilities were measured with an applied voltage of 10 kV (157 V/cm) at 25 ± 0.1°C. Three measurements were made. The relative standard deviations were better than 0.5%.

Buffers were prepared in deionized water (Milli-Q system; Millipore, Bedford, MA, U.S.A.). Borax concentrations are expressed as equivalent borate, for reasons which are explained in the text.

RESULTS AND DISCUSSION

The electroosmotic mobility was measured in four buffer systems (Fig. 3): Tris–HCl, H_3BO_3–NaOH, $Na_2B_4O_7$–NaOH and $Na_2B_4O_7$–HCl. The results confirm that boric acid and borax are funtionally similar when titrated with a base [24]:

$$Na_2B_4O_7 + 7\,H_2O = 2\,Na^+\,B(OH)_4^- + 2\,H_3BO_3 = 2\,NaOH + 4\,H_3BO_3$$

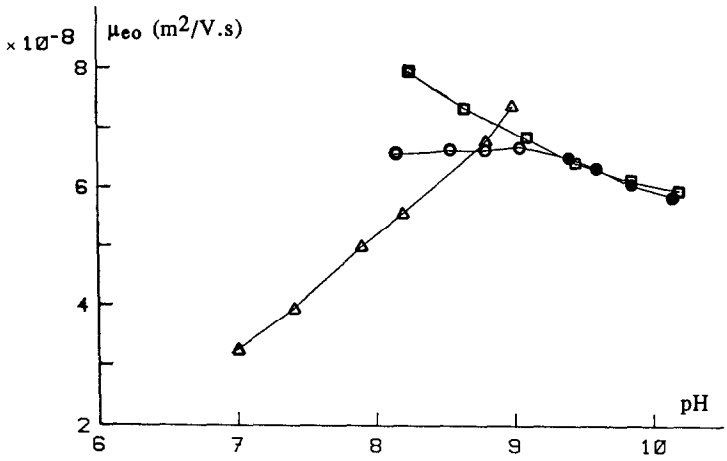

Fig. 3. Influence of buffer pH on electroosmotic mobility. △ = Tris–HCl; □ = boric acid–NaOH ○ = borax–HCl; ● = borax–NaOH. Buffer concentration: 50 m*M*.

For this reason, we express borax concentrations as their boric acid equivalent (*e.g.*, 25 m*M* borax buffer is equivalent to 100 m*M* boric acid buffer).

Three different cases remain to relate pH and ionic strength during titration, assuming that for these weak electrolytes the ion concentrations are equal to the analytical concentrations of the titrant:

(a) $Tris + HCl = TrisH^+ + Cl^-$
(b) $H_3BO_3 + NaOH = Na^+ + B(OH)_4^-$
(c) $2\ Na^+ + 2B(OH)_4^- + HCl = 2\ Na^+ + B(OH)_4^- + Cl^- + H_3BO_3$

In case (a) a decrease in pH is accompanied by an increase in the number of ions, and thus of the ionic strength. Both effects are cooperative in producing a relatively strong dependence of the electroosmotic mobility on the pH. In case (b), however, increasing the pH will result in an increase in the ionic strength. Apparently, the ionic strength effect is stronger than the "pure" pH effect, resulting in an inverted dependence of the electroosmotic flow on the pH. The borax–NaOH case is similar as it can be considered as a boric acid–NaOH system that has already been partially neutralized. All the cited examples, showing lower electroosmotic mobility at higher pH, belong to this category. Case (c) is interesting, as the amount of ions remains constant during neutralization. Provided that wall interactions do not differ too much from the boric acid–NaOH system, the data confirm that, indeed, the mobility increases slightly with pH, as expected. In this pH range however, the effect is so moderate that it is easily overcompensated by ionic strength effects.

When differences in the amount of titrant are compensated for by adding a calculated amount of salt, the anomalous decrease in mobility with pH disappears and a fairly flat profile is obtained (Fig. 4). The confusion between constant concentration and constant ionic strength explains the anomaly. In published studies, the use of a pH scale with fixed ionic strength [3] is exceptional. In most of the op-

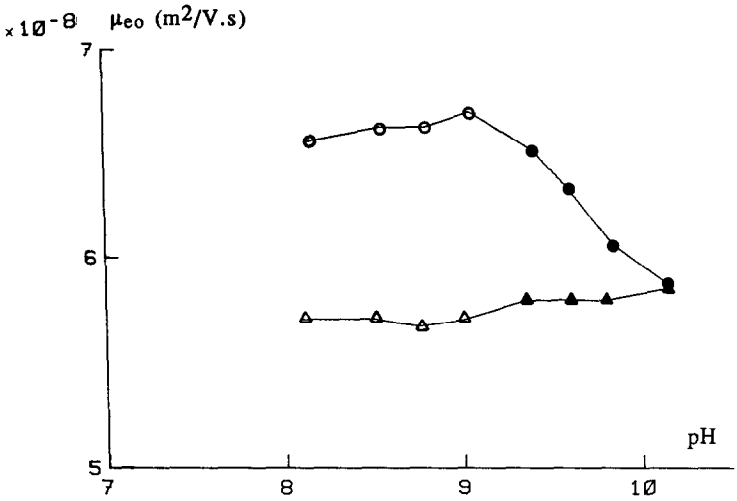

Fig. 4. Influence of added salt on electroosmotic mobility: (●) borax–NaOH and (○) borax–HCl at constant concentration (50 m*M*); (▲) borax–NaOH and (△) borax–HCl at constant ionic strength (50 m*M*).

timization reports, where the pH change was limited to only a few units, constant-concentration buffers [1,2,4,6,13,19–23] were used, often by mixing two constituents, *e.g.*, primary and secondary phosphate, in varying ratios. There may be valid practical reasons for doing so, but a numerical example demonstrates the possible pitfall of such a procedure. A 100 m*M* borate buffer, 5% neutralized with NaOH (considering the first neutralization step only), has a pH of 8.1 and an ionic strength of 5 m*M*. Doubling the concentration will double the ionic strength. However, increasing the pH of the 100 m*M* solution by 1 unit will require a degree of neutralization of *ca.* 24%, resulting in an ionic strength of 24 m*M*, nearly a five-fold increase. This explains why ionic strength effects are more prominent through pH than through buffer concentration, as we aboserved in our own optimization set-up [1].

CONCLUSIONS

When using constant-concentration buffers, decreased electroosmotic mobility can be observed at higher pH values. This effect is attributable to the ionic strength and will be observed with buffers obtaind by titrating a weak acid with a strong base. To obtain an increased electroosmotic flow, weak base–strong acid-type buffers are to be preferred. If, on the other hand, pure pH effects are desired, buffers at constant ionic strength, rather than at constant concentration, should be used.

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